CODING FORM FOR SRC INDEXING **REVISED 10/15/86**

Microfiche No.	
	OTS0528814

New Doc I.D.	Old Doc I.D.
86-910000638	

Date Produced	Date Received	TSCA section
10/19/77	1/24/91	8D

Submitting Organization
W.R. GRACE & COMPANY

Contractor
LITTON BIONETICS INC

Document Title
MUTAGENICITY EVALUATION OF 10055-49-2 IN THE MOUSE LYMPHOMA ASSAY (FINAL REPORT) WITH COVER LETTER DATED 011691

Chemical Category
TOLUENE DIISOCYANATE (26471-62-5)

CONTAINS NO CBI

GRACE 86-910000638

Joseph W. Raksis, Vice President
Research Division

W.R. Grace & Co.-Conn.
7379 Route 32
Columbia, Maryland 21044

(301) 531-4331

January 16, 1991

Environmental Protection Agency
Document Processing Center (TS-790)
Room L-100
Office of Toxic Substances
401 "M" Street S.W.
Washington, D.C. 20460

Attn: Health and Safety Reporting Rule (Notification/Reporting)

Please find attached 8(d) health and safety reports for mixtures processed containing toluene diisocyanate (CAS #26471-62-5), 4,4-Diphenylmethane diisocyanate (CAS #101-68-8) and 1,6-Diisocyanatohexane (CAS #822-06-0). Grace is submitting these reports for late filing since their submittal may have been subject to the isocyanates 10-year call-in of June 1, 1987.

We have reason to believe that some of these reports may have previously been submitted to EPA as attachments to PMN submissions. However, Grace is filing them as a precautionary measure to insure EPA's receipt.

These reports are being submitted for:

W. R. Grace & Co.-Conn.
Washington Research Center
7379 Route 32
Columbia, MD 21044

Sincerely,

J. W. Raksis

A:\JR91-013/lw

Attachments - 20

86910000638

PD 1-16-91

3001 Liquid

CONTAINS NO CBI

Toluene Diisocyanate
(CAS # 26471-62-5)
1,6-Diisocyanatohexane
(CAS # 822-06-0

MUTAGENICITY EVALUATION

OF

10055-49-2

IN THE MOUSE LYMPHOMA ASSAY
FINAL REPORT

SUBMITTED TO

W.R. GRACE AND COMPANY
7379 ROUTE 32
COLUMBIA, MARYLAND 21044

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20839

Litton BIONETICS

TABLE OF CONTENTS

EVALUATION SUMMARY

The test sample, 10055-49-2, did not produce any clear evidence of mutation at the TK locus of the L5178Y mouse lymphoma cells under the conditions of this assay.

SPONSOR: W.R. Grace and Company

MATERIAL: 10055-49-2

SUBJECT: FINAL REPORT L5178Y MOUSE LYMPHOMA MUTAGENICITY ASSAY

1. OBJECTIVE

The objective of this study was to evaluate 10055-49-2 for specific locus forward mutation induction in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay.

2. MATERIALS

A. Test Compound

1. Date Received: March 25, 1977

2. Description: Yellow semi-solid

B. Indicator Cells

The Fischer mouse lymphoma cell line used in this study was derived from L5178Y. The cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BUdR) sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR.

C. Media

The cells were maintained in Fischer's Medium for Leukemic Cells of Mice with 10% horse serum and sodium pyruvate. Cloning medium consisted of Fischer's medium with 20% horse serum, sodium pyruvate, and 0.37% agar. Selection medium was made from cloning medium by the addition of 5.0 mg of BUdR to 100 ml of cloning medium.

D. Control Compounds

1. Negative Control

The solvent in which the test compound was dissolved was used as a negative control and is designated as solvent control in the data table. The actual solvent is listed in the Results Section.

2. MATERIALS (Continued)

D. Control Compounds

2. Positive Controls

Ethylmethanesulfonate (EMS), which induces mutation by base-pair substitution, was dissolved in culture medium and used as a positive control for the nonactivation studies at a final concentration of 5 µl/ml.

Dimethylnitrosamine (DMN), which requires metabolic biotransformation by microsomal enzymes, was used as a positive control substance for the activation studies at a final concentration of 5 µl/ml.

3. EXPERIMENTAL DESIGN

A. Toxicity

The solubility, toxicity, and doses for all chemicals were determined prior to screening. The effect of each chemical on the survival of the indicator cells was determined by exposing the cells to a wide range of chemical concentrations in complete growth medium. Toxicity was measured as loss in growth potential of the cells induced by a four-hour exposure to the chemical followed by a 24-hour expression period in growth medium. A minimum of four doses was selected from the range of concentration by using the highest dose that showed no loss in growth potential as the penultimate dose and by bracketing this with one higher dose and at least two lower doses. Toxicity produced by chemical treatment was monitored during the experiment.

B. Assays

1. Nonactivation Assay

The procedure used is a modification of that reported by Clive and Spector (Mutation Research, 31:17-29, 1975). Prior to each treatment, cells were cleansed of spontaneous TK-/- by growing them in a medium containing thymidine, hypoxanthine, methotrexate, and glycine (THMG). This medium permits the survival of only those cells that produce the enzyme thymidine kinase, and can therefore utilize the exogenous thymidine from the medium. The test compound was added to the cleansed cells in growth medium at the predetermined doses for four hours. The mutagenized cells were washed, fed, and allowed to express in growth medium for three days. At the end of this expression period, TK-/- mutants were detected by cloning the cells in the selection medium for ten days. Surviving cell populations were determined by plating diluted aliquots in nonselective growth medium.

3. EXPERIMENTAL DESIGN (Continued)

B. Assays

2. Activation Assay

The activation assay differs from the nonactivation assay in the following manner only. Two milliliters of the reaction mixture were added to 10 ml of growth medium. The desired number of cleansed cells was added to this mixture, and the flask was incubated on a rotary shaker for four hours. The incubation period was terminated by washing the cells twice with growth medium. The washed mutagenized cells were then allowed to express for three days and were cloned as indicated for the nonactivated cells.

C. Preparation of 9,000 x g Supernatant

Male random bred mice were killed by cranial blow, decapitated, and bled. The liver was immediately dissected from the animal using aseptic technique and placed in ice-cold 0.25M sucrose buffered with Tris buffer at a pH of 7.4. When an adequate number of livers had been collected, they were washed twice with fresh buffered sucrose and completely homogenized. The homogenate was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from this centrifuged sample was retained and frozen at -80C until used in the activation system. This microsome preparation was added to a "core" reaction mixture to form the activation system described below:

Component	Final Concentration/ml
1. TPN (sodium salt)	6 µmoles
2. Isocitric acid	35 µmoles
3. Tris buffer, pH 7.4	28 µmoles
4. $MgCl_2$	2 µmoles
5. Homogenate fraction equivalent to 25 mg of wet tissue	

D. Screening

A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. Colonies were counted on an electronic colony counter that resolves all colonies greater than 200 microns in diameter.

4. RESULTS

The data presented in the following tables show the concentrations of the test compound employed, the number of mutant clones obtained, the surviving populations after the expression period, and the calculated mutation frequencies.

4.__SUMMARY_OF_MOUSE_LYMPHOMA_TL5178Y1_RESULTS EITHON BIONETICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: WR Grace 10055-49-2
B. SOLVENT: DMSO
C. TEST DATE: 07/25/77
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) OR NANOLITERS(NL) PER MILLILITER.

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10.E-6)
NONACTIVATION											
SOLVENT CONTROL	---	---	8.4	12.0	13.7	100.0	10.0	192.0	100.0	100.0	0.0521
NEGATIVE CONTROL	---	---	14.2	12.2	11.8	148.0	7.0	170.0	88.5	131.1	0.0412
DMN .5UL/ML	---	---	5.0	12.4	14.4	64.7	358.0	85.0	44.3	28.6	4.2118
TEST COMPOUND											
0.50000 UL/ML	---	---	3.6	15.8	8.8	30.6	22.0	98.0	51.0	15.6	0.2245
1.00000 UL/ML	---	---	1.8	6.0	5.2	6.9	33.0	153.0	79.7	5.5	0.2157
2.50000 UL/ML	---	---	3.8	10.0	10.6	23.2	6.0	167.0	87.0	20.1	0.0359
5.00000 UL/ML	---	---	3.2	6.0	10.6	10.2	11.0	179.0	93.2	9.5	0.0615
10.00000 UL/ML	---	---	1.0	4.0	5.6	4.9	35.0	159.0	82.8	4.0	0.2201
ACTIVATION											
SOLVENT CONTROL	MOUSE	LIVER	6.6	15.4	10.2	100.0	10.0	230.0	100.0	100.0	0.0435
NEGATIVE CONTROL	MOUSE	LIVER	6.0	15.6	11.8	106.5	10.0	145.0	63.0	67.2	0.0690
DMN .5UL/ML	MOUSE	LIVER	7.0	5.2	10.0	35.1	154.0	97.0	42.2	14.8	1.5876
TEST COMPOUND											
0.50000 UL/ML	MOUSE	LIVER	5.6	6.2	12.6	42.2	45.0	150.0	65.2	27.5	0.3000
1.00000 UL/ML	MOUSE	LIVER	2.6	4.0	5.0	5.9	187.0	136.0	59.1	3.5	1.3750
2.50000 UL/ML	MOUSE	LIVER	9.0	8.0	12.6	87.5	16.0	189.0	82.2	71.9	0.0847
5.00000 UL/ML	MOUSE	LIVER	5.2	11.4	14.4	82.3	14.0	161.0	70.0	57.6	0.0870
10.00000 UL/ML	MOUSE	LIVER	1.0	0.4	2.8	2.9	30.0	68.0	29.6	0.9	0.4412

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** (MUTANT CLONES / VIABLE CLONES) X 10.E-6

TABLE 2

SUMMARY OF MOUSE LYMPHOMA ASSAY RESULTS — LITTON BIONETICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: WR Grace 10055-49-2
B. SOLVENT: DMSO
C. TEST DATE: 09/11/77
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) OR NANOLITERS(NL) PER MILLILITER.

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** X 10.E-4
NONACTIVATION											
SOLVENT CONTROL	---	---	14.4	9.8	15.0	100.0	8.5	171.5	100.0	100.0	0.0496
NEGATIVE CONTROL	---	---	19.0	8.0	15.6	112.0	5.0	178.0	103.8	116.3	0.0281
EMS .5UL/ML	---	---	7.4	7.4	11.6	30.0	104.0	46.0	26.8	8.0	2.2609
TEST COMPOUND											
0.50000 UL/ML	---	---	7.4	9.4	12.4	40.7	19.0	128.0	74.6	30.4	0.1484
0.75000 UL/ML	---	---	4.4	5.8	11.8	14.2	19.0	157.0	91.5	13.0	0.1210
1.00000 UL/ML	---	---	4.8	12.2	7.8	21.6	17.0	149.0	86.9	18.7	0.1141
2.00000 UL/ML	---	---	1.0	6.2	10.2	9.0	24.0	214.0	124.8	11.2	0.1121
4.00000 UL/ML	---	---	1.6	5.2	6.0	4.5					
ACTIVATION											
SOLVENT CONTROL	MOUSE	LIVER	11.5	9.5	12.0	100.0	8.0	193.5	100.0	100.0	0.0413
NEGATIVE CONTROL	MOUSE	LIVER	11.6	8.0	16.0	113.3	1.0	180.0	93.0	105.4	0.0056
DMN .5UL/ML	MOUSE	LIVER	7.0	5.8	7.2	22.3	84.0	46.0	23.8	5.3	1.8261
TEST COMPOUND											
1.00000 UL/ML	MOUSE	LIVER	7.2	6.2	11.0	37.5	27.0	168.0	86.8	32.5	0.1607
2.00000 UL/ML	MOUSE	LIVER	2.6	10.6	10.2	25.2	20.0	146.0	75.5	19.0	0.1370

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** (MUTANT CLONES / VIABLE CLONES) X 10.E-4

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

A. Toxicity

The test substance, 10055-49-2, was examined for cell toxicity in a series of concentrations ranging from 0.5 µl/ml to 10 µl/ml. There was some toxicity evident at the higher concentrations as indicated in the daily suspension counts. However, the overall Percent Relative Growth was not significantly affected. The test chemical formed a white, oily precipitate as soon as it was placed in the test medium. The precipitated material was gummy and stuck to objects used to disperse it. Variable survival evidenced in the test data were probably more the result of cell loss in the precipitate than by actual toxicity of the substance.

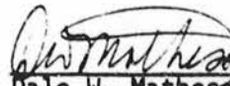
B. Test Results

Two separate runs of this compound were conducted (Tables 1 and 2). The data was quite variable in the initial test run (Table 1), but no indication of dose-related mutation was observed. A single high frequency was observed at the 1 µl/ml concentration in the activation test. The second test was initiated to define the confidence in the unusual high value (Table 2). The results from the second test were considered negative at the 1 µl/ml and 2 µl/ml concentrations with activation and at all concentrations in the nonactivation test.

C. Conclusions

The test sample, 10055-49-2, did not produce any clear evidence of mutation induction at the TK locus of the L5178Y mouse lymphoma cells under the conditions of this evaluation.

Submitted by:



10/19/77

Dale W. Matheson, Ph.D. Date
Section Chief
Mammalian Genetics
Department of Molecular
Toxicology

Reviewed by:

10/17/77

David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology

Litton BIONETICS

6. CRITERIA USED IN THE EVALUATION

Several criteria have been established which, if met, provide a basis for declaring a material genetically active in the Mouse Lymphoma Assay. These criteria are derived from a historical data base and are helpful in maintaining uniformity in evaluations from material to material and run to run. While these criteria are reasonably objective, a certain amount of flexibility may be required in making the final evaluation since absolute criteria may not be applicable to all biological data.

A compound is considered mutagenic in the Mouse Lymphoma Assay if:

a. A dose response relationship is observed over three of the four dose levels employed.

b. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value.

c. The solvent control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on the concurrent solvent control value run with the experiment in question. Positive control values are not used as reference points, but are included to ensure the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

As the data base on the assay increases, the evaluation criteria can be expected to become more firmly established.

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 4 (Month) 10 (Day) 91 (Year) Barbara Smith Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp